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Extended Abstracts of the Lectures

SESSION I

Genetics of IBD: Unique Aspects of Early-onset Gut Inflammation

Moderator: Subra Kugathasan

GENE DISCOVERY IN IBD: A DECADE OF PROGRESS

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The author reports no conflicts of interest.

Striking progress has been made in identifying genetic determinants of disease susceptibility and phenotype in both forms of IBDs, Crohn disease and ulcerative colitis, and there is real optimism that the success in gene discovery will translate to progress in clinical management by improving accuracy of diagnosis, predictions of outcomes, and responses to medical therapy, as well as by leading to the development of novel therapies.

Studies of concordance rates in twin pairs and in multiply affected families provided the stimulus for detailed molecular studies. The derived heritability rates from twin concordance data in Crohn disease are at least equivalent to those in other polygenic diseases, which have been the subject of intensive investigation. These data led to the development, by investigators throughout the world, of detailed repositories of DNA and clinical material from patients with IBD, multiply affected families, and population-based control subjects for linkage and association studies.

In 1996, 2 high-impact publications reported the first genomewide scans in IBD using nonparametric-linkage analysis (analysis of patterns of chromosomal marker sharing in multiply affected families or relative pairs). The identification of the IBD1 locus on chromosome 16, implicated in Crohn disease, was replicated rapidly, and these papers remain widely regarded as landmarks in complex disease genetics. Supplementary data from the United Kingdom provided the first molecular evidence that Crohn disease and ulcerative colitis are related polygenic diseases, sharing some but not all susceptibility loci. These initial studies were complemented rapidly by genomewide linkage studies involving families and affected relative pairs throughout the world, leading to the identification of other chromosomal subregions requiring detailed investigation.

Application of a classical fine mapping and positional cloning strategy enabled Hugot et al to identify the IBD1 gene as nucleotide-binding oligomerization domain 2/caspase recruitment domain 15 (NOD2/CARD15), an observation again rapidly replicated by other investigators. The importance of germline variation of NOD2/CARD15 in Crohn disease, the putative function of the NOD2/CARD15 protein, and the mechanisms whereby NOD2/CARD15 dysfunction gives rise to the inappropriate intestinal inflammation of Crohn disease are still under intense investigation. It is apparent, from data generated both in vitro and in vivo, that NOD2/CARD15 is an intracellular ligand for muramyl dipeptide, a motif common to gram-positive and gram-negative bacterial cell walls. These observations heavily implicate primary dysfunction of the innate immune system in IBD pathogenesis.

Progress has been made in identifying genetic determinants within other subchromosomal regions implicated by linkage analyses, notably the IBD2 locus. However, within the last 2 years, the technique of whole genome association scanning has gained precedence over linkage studies. This technique, involving the comparison of the genetic profiles of affected individuals with controls, requires rigorous, well-powered studies to be designed and executed. Remarkably, many of the most successful discoveries in complex diseases relate to Crohn disease. Recent discoveries using this technique have included the identification of the interleukin-23 receptor gene, *ATG16L1* gene, and immunity-related GTPase family M gene as determinants of susceptibility to Crohn disease. Each observation has been replicated in a number of data sets, providing considerable support for the robust nature of this methodology and the importance of these observations in understanding disease pathogenesis.

Many studies are ongoing, including initial genomewide association studies in ulcerative colitis and in childhood-onset IBD. These data will provide a map of the genetic architecture of IBD, thereby providing insight into pathogenesis, disease heterogeneity, and novel therapies.

Key References

Gaya DR, Russell RK, Nimmo E, Satsangi J. Genetics of inflammatory bowel diseases: lessons for complex Tdiseases? *Lancet* 2006;367: 1271–84.

The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–7.
Cho JH, Weaver CT. The genetics of inflammatory bowel disease. *Gastroenterology* 2007;133:1327–39.

EPIGENETICS AND COMPLEX HUMAN DISEASE: IS THERE A ROLE IN IBD?

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The author reports no conflicts of interest.

During the last decade, there has been enormous progress in the identification of genes involved in various disease processes in concert with the sequencing of the human genome. This has involved studies of genetic linkage, linkage disequilibrium, and genotype association. This approach has been successful in studies of IBD, and these discoveries will be reviewed briefly. These studies have focused almost entirely on genetic as opposed to epigenetic abnormalities and on variations that are inherited rather than of de novo origin. In our studies of the role of genomic imprinting in disease, we have become cognizant of the role of epigenetics and of de novo events in the etiology of disease. For example, in the case of Angelman syndrome, patients may have large de novo genetic deletions, uniparental disomy (an epigenetic abnormality), imprinting defects associated with small deletions of an imprinting center, imprinting defects associated with no nucleotide sequence abnormality, and mutations in the Angelman gene (*UBE3A*). This experience has led us to develop a disease model that emphasizes the potential role for epigenetics and de novo events. We have referred to this as a mixed epigenetic and genetic and mixed de novo and inherited (MEGDI) model for human disease. This model is exemplified by disorders such as Angelman syndrome, Prader-Willi syndrome, Beckwith-Weidemann syndrome, and various forms of pseudohypoparathyroidism caused by abnormalities of the *GNAS* gene cluster. We are studying the possibility that the MEGDI model may provide an important hypothesis for designing research strategies on disorders such as autism and schizophrenia. Recently, the National Institutes of Health Roadmap Initiative has identified epigenetics as an important topic for additional research emphasis. The ways in which epigenetics may contribute to the pathogenesis of IBD will be discussed.

Key References

Hatchwell E, Greally JM. The potential role of epigenomic dysregulation in complex human disease. *Trends Genet* 2007;23:588–95.
Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* 2007;447:433–40.
Jiang YH, Bressler J, Beaudet AL. Epigenetics and human disease. *Annu Rev Genomics Hum Genet* 2004;5:479–510.

GENETIC STUDY DESIGN ISSUES: PROBLEMS AND PITFALLS

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The author reports no conflicts of interest.

Most chronic diseases have complex etiologies. Twin and family studies point to both genetics and environment in the pathogenesis of disorders such as type 1 diabetes mellitus, schizophrenia, multiple sclerosis, and IBD. Indeed, the disease itself may have arisen from a number of different but limited underlying pathological pathways. To understand the pathogenesis of these complex disorders, we must lay out the genetic underpinnings and use tight diagnostic criteria. It makes sense to tease out the genetic effects before understanding how different environments may interact with specific genetic components that lead to disease. A parsimonious hypothesis is that the environment may act on the same pathways already perturbed by the inheritance of a constellation of susceptibility gene variants. Thus, a global appreciation of the genetic basis of a complex disease will give a rich insight into the pathogenesis of the disease and possibly the environmental agents that could influence the same pathway. In turn, this will lead to better diagnostic groups and tailor-made strategies for prevention and eventual therapy for our patients. The dawn of a clearer understanding of complex diseases is near and this will revolutionize medicine.

But how did we get here? The theory for whole genome association was firmly established by 1996. Various studies have shown the increased power of genetic association, as opposed to linkage, with large sample sizes. The molecular technology was lacking and the statistical issues, especially with respect to multiple testing, were underdeveloped. These missing methodologies soon appeared on the scene, however. Because of the inherent ease of collection and a better understanding of heterogeneity within a population (structure), case-control studies have become the norm. The HapMap project allowed us to identify a substantial number of single nucleotide polymorphisms, a proportion of which can be typed as proxies for the majority of the genomic markers, owing to physical proximity and statistical correlation (linkage disequilibrium). The reduced costs for typing just proxies, coupled with vast database resources and new technologies from Illumina and Affymetrix, transformed theory into reality.

How do we successfully carry out a genomewide association study into IBD and other diseases? And what do we do with the results? How do we go from genetic to etiologic variants and find out what they do? The most important consideration is to make sure that the study is well powered. There are also several issues to consider after genotyping in terms of quality control. The issue of missing data is a reality, but can we impute the data? And what does that do to biased outcomes? There are advantages and disadvantages to creating haplotypes from adjacent markers, in an attempt to understand the chromosomal effects. Next, how do we analyze 2 or more loci together and will this be a more powerful approach? Finally, do our results hold true in another sample set? Replication is probably the most important aspect of a successful genetic study. These and other methodological issues will be discussed in greater detail.

Key References

Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996;273:1516–7.

Balding DJ. A tutorial on statistical methods for population association studies. *Nat Rev Genet* 2006;7:781–91.

Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.

DYSFUNCTIONAL GENOMICS: TOWARD AN INTEGRATIVE BIOLOGY OF DISEASE AND HEALTH—APPLICATION TO IBDs

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The author reports no conflicts of interest.

Integrative biology is an exciting approach to the combinatorial application of knowledge derived from all provinces of structural and functional biology, chemistry, and physics that can be used to model the dynamic states and performance of complex biological systems. Harnessing knowledge of gene–gene–function relationships derived from multiple species across whole genomes provides a powerful matrix over which various disease processes and disease to gene–gene variant and mutation relationships can be analyzed. While still early in a nascent in concert with the sequencing of the human genome, even in the presence of mutations, the robustness of biological systems derived from compensatory process relationships should offer new approaches for the minimization of disease phenotype and the maximization of healthy performance. For affected individuals, knowledge of individual genetics, functional genomic, epigenomic, microbiomic states, environmental factors—combined with an increasingly high-resolution view of molecular, cellular, and organ system biology—should both provide improved knowledge of disease mechanism and offer predictive methods for suppressing disease progression and clinical phenotype. We will present how a variety of data types (known gene–disease associations, protein function prediction, expression patterns, cell type–specific gene expression, metagenomics and orthologenomics) can be used to build linked disease-process and clinical care–process models that can be used to dissect, study, and model disease progression, therapeutic optimization, and therapeutic discovery in Crohn disease (CD).

We are using a variety of methods to increase connectivity across data, including conventional relational data structures; object modeling of biological entities, events, and systems; and linking biological ontologies through novel semantic Web technology–based assertions between data entities that have specific relationship types that are describable from ontologies. The systems that we are developing use the National Library of Medicine's Unified Medical Language System and the data sources that it encompasses include Systematized Nomenclature of Medicine—Clinical Terms, Online Mendelian Inheritance in Man, and Mouse Genome Informatics phenotype data for core disease feature representations (Fig. 1). All disease-related information is then associated to genes, proteins, biological networks, human genetic variation, and potential implications, pathways, and gene-expression data. GATACA integrates associations of genes, gene ontologies, gene interactions, and gene pathways with clinical abnormalities, clinical features, phenotypes, disease entities, and anatomical entities to form a disease-associated gene network. PolyDoms takes the gene network and examines all known gene variations and mutations for predicted functional impact of these gene polymorphisms on known protein structures, domains, mutation sites, and conservation scores. Similarly, new functionalities include performing polymorphism impact analyses of gene regulatory regions in promoters and other important gene features. The systems that we have developed to perform these analyses can be accessed at *http://polydoms.cchmc.org* and *http://genometrafac.cchmc.org*. For example, a preliminary cluster-based analysis of clinical, genetic, and serological parameters in patients with pediatric-onset CD was performed to better understand and predict which patients with CD are at high risk for complications. Data corresponding to disease presentation, progression, complications, and stage-specific requirements for surgery were obtained at diagnosis and during follow-up for a cohort of 254 patients with pediatric-onset CD at a single center. Patients were studied for a median of 4 years from diagnosis. Patients are genotyped for the 3 predominant CARD15 susceptibility single nucleotide polymorphisms, and serum levels of anti-*Saccharomyces cerevisiae* antibodies, Cbir1, I2, outer membrane protein

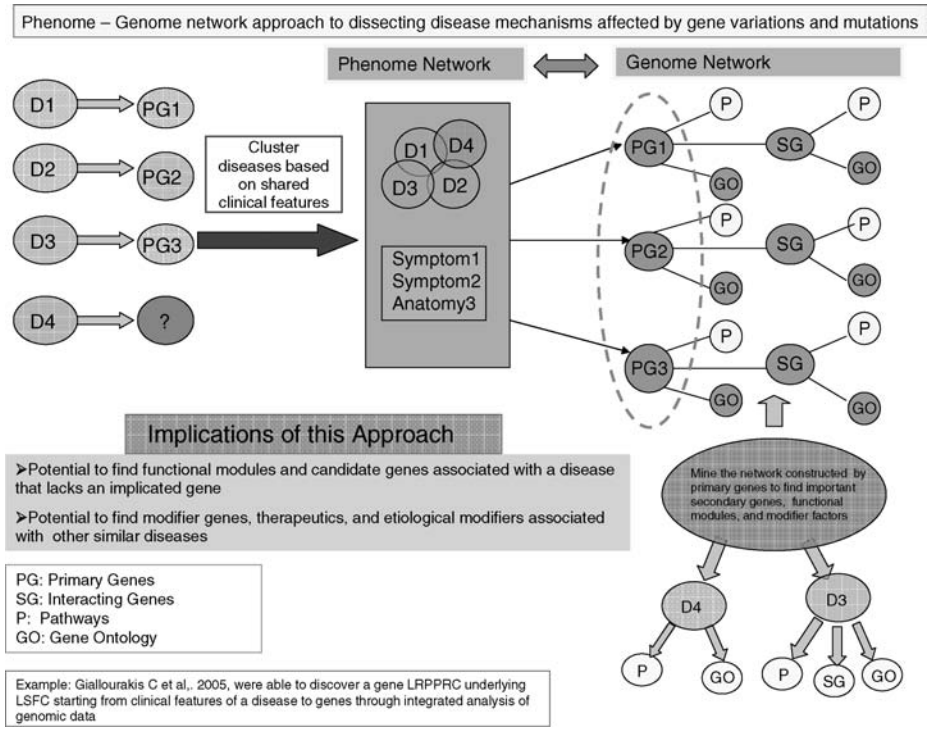

FIG. 1.

C, and perinuclear antineutrophil cytoplasmic antibody were determined. Data transformations were made according to disease process and clinical care process models for multicenter data aggregation and subsequent simulation and dynamic modeling efforts. The correlated patients and clinical conditions thus detect groups of subjects who share similar patterns of selected clinical, genetic, and serological findings. Many examples of focused systems representations of IBD and other chronic inflammatory disease processes and mechanisms will be presented.

Key References

Giallourakis C, Henson C, Reich M, et al. Disease gene discovery through integrative genomics. *Annu Rev Genomics Hum Genet* 2005;6:381–406.

Mootha VK, Lepage P, Miller K, et al. Identification of a gene causing human cytochrome c oxidase deficiency by integrative genomics. *Proc Natl Acad Sci USA* 2003; 100:605–10.

GENOTYPE/PHENOTYPE CORRELATIONS AND IMPLICATIONS FOR CLINICAL PRACTICE

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The author reports no conflicts of interest.

Inflammatory bowel disease (IBD) is a term that defines chronic inflammatory diseases of the gastrointestinal tract that result in lifelong symptoms and the need for significant medical and surgical therapy. Approximately 20% to 25% of patients are diagnosed under the age of 18. Although there are similarities between adult-onset and early-onset IBD, there are also significant clinical differences, and these differences are correlated with age of onset. Phenotypic differences in pediatric ulcerative colitis (UC) as compared with adult-onset UC include a greater proportion of extensive UC and the rare occurrence of proctitis; also, gastric and duodenal inflammation appears to be more prevalent in pediatric UC. In early-onset Crohn disease (CD), there is a preponderance of colonic CD in the very young (<8 years of age), a higher proportion of inflammatory disease behavior and less perianal disease, a higher male-to-female ratio among those with prepubertal CD, a higher proportion of Jewish individuals, and higher anti-*Saccharomyces cerevisiae* antibodies seropositivity rates, as well as speculation about a more rapid and progressive disease course, although data supporting this point are somewhat limited.

The etiology of IBD is still unknown, but it is thought to occur as a result of exposure to an environmental triggering event in a genetically susceptible host. There is a worldwide effort under way aimed at identifying the genetic variants responsible for such susceptibility. Although significant progress has been made, there is still much work to be done in fully identifying all of the

important genetic variants, particularly for UC. For most complex disorders, as age of onset increases environmental variance increases, while heritability decreases, making the study of the young population particularly desirable. For IBD, there are several lines of evidence to support efforts to study the pediatric population. In early-onset IBD, there is a higher proportion of those reporting a family history of IBD (particularly among the youngest affected), increased linkage evidence at the IBD1 and IBD5 loci, and a mutation ''dose'' effect for nucleotide-binding oligomerization domain 2 in early-onset disease. Therefore, studying individuals with pediatric IBD should result in an increased ability to identify genetic variants that may be difficult to find in adults with IBD. Such variants may turn out to be unique to the pediatric population or replicated in the adult population. Collaborative genomewide association studies are being initiated in the pediatric population to answer these questions.

With the identification of all of the important genetic variants in IBD, the task will turn to understanding how these variants affect the clinical course of disease. Most studies evaluating genotype–phenotype relations have been performed in adult populations, but pediatric studies are increasingly common. There is evidence that genotype and allele frequencies may be different in the early-onset population for some genes (eg, nucleotide-binding oligomerization domain 2) and similar to the adult populations for others (*IL23R, IBD5*), although pediatric studies are somewhat sparse and underpowered to date. Data also suggest a protective effect of the discs, large homolog 5 (*Drosophila*) gene in young females, perhaps partially explaining the higher male-to-female ratio in early-onset CD. There is some evidence in pediatric studies to suggest that genetic variants in nucleotide-binding oligomerization domain 2 are associated with markers of CD severity. Based on existing knowledge, it is speculated that genetic and possibly serological markers are correlated with disease location, behavior, and other clinical outcomes. Further studies involving larger, collaborative efforts in the early-onset population also should allow a more accurate evaluation of these markers and outcomes. The Montreal Classification already has made significant movement toward the concept that early-onset IBD reflects a unique population in terms of clinical and genetic profiles with the introduction of a new age of onset classification for those diagnosed with CD at 16 years and younger.

In addition to using genetic markers to evaluate disease course, the ultimate goal of the research program in the genetics of IBD is to identify those at risk for IBD and to prevent disease from developing. The pediatric population is uniquely suited to these efforts. Research in the pediatric and young adult population is under way to prospectively follow high-risk individuals (eg, healthy siblings of affected patients) to attempt to identify the genetic, environmental, and microbial risk factors in the ''at risk'' population (GEM Project, Crohn's and Colitis Foundation of Canada).

Key References

Babusukumar U, Wang T, McGuire E, et al. Contribution of OCTN variants within the IBD5 locus to pediatric onset Crohn's disease. *Am J Gastroenterol* 2006;101:1354–61.

Biank V, Broeckel U, Kugathasan S. Pediatric inflammatory bowel disease: clinical and molecular genetics. *Inflamm Bowel Dis* 2007; 13:1430–8.

Paul T, Birnbaum A, Pal DK, et al. Distinct phenotype of early childhood inflammatory bowel disease. *J Clin Gastroenterol* 2006;40: 583–6.

Russell RK, Drummond HE, Nimmo EE, et al. Genotype-phenotype analysis in childhood-onset Crohn's disease: NOD2/CARD15 variants consistently predict phenotypic characteristics of severe disease. *Inflamm Bowel Dis* 2005;11:955–64.

SESSION II

Genetic Variability and Environmental Stress

Moderator: Richard Grand

WAR AND PEACE AT THE INTESTINAL EPITHELIAL SURFACE: AN INTEGRATED VIEW OF BACTERIAL COMMENSALISM VERSUS BACTERIAL PATHOGENICITY

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The author reports no conflicts of interest.

Mammals, particularly humans, maintain a balanced relationship with their microbiota. This is obvious in the gut, particularly in the distal ileum and in the colon, where the commensal flora can reach an ''astronomic'' density: 10^{11} bacteria per gram of stool belonging to at least 500 different species, largely anaerobic, which are dominated by *Bacteroides*, *Escherichia coli*, *Clostridia*, *Streptococcus,* and *Lactobacillus*. In spite of this massive quantity of luminal bacteria, the fetal gut is sterile, with bacterial colonization beginning immediately after birth. Enterobacteriaceae and bifidobacteria are among the pioneering colonizers, although significant differences exist between breast-fed and formula-fed infants. These pioneering microbes can prevent further colonization by certain species by establishing a protective barrier, and they can modulate host epithelial gene expression, causing modification of epithelial surfaces (ie, fucosylation of surface glycolipids and glycoproteins) to create a suitable environment for additional species to settle in. Once established, the gut microbiota comprises 10 times more microorganisms than the number of cells forming our body, and have a collective metabolic activity equal to an organ such as the liver. One may therefore consider that we are ''human–bacterial'' hybrids, such that the human genome will not be completed until the metagenome of its commensal flora is deciphered. This microbiota has several functions: protection against invaders (barrier effect), particularly pathogens, by competition for nutrients and receptors, and by production of various antimicrobial factors; structural functions corresponding to strengthening of the epithelial/mucosal barrier, either by reinforcing cell junctions or by stimulating the development of the mucosal immune system and its activation (ie, production of immunoglobulin A); and metabolic functions including synthesis of vitamins, enzymatic hydrolysis of nondigestible vegetal polysaccharides, direct supply of short-chain fatty acids that represent 50% of the energy metabolized by colonic cells, and fat storage.

Permanent exposure to this microbiota and accidental exposure to pathogenic microorganisms have forged the intestinal immune system under the constraint that it needs to perform accurate interpretation of its microbial microenvironment to discriminate between permanently established commensal bacteria and episodic pathogens. The intestinal epithelium has clearly evolved to resist microbial aggression by establishing an integrated system of defense comprising a mucus layer that is constantly mobilized by intestinal movements (ie, peristalsis), a sealed barrier combined with the production of antimicrobial molecules by intestinal epithelial cells, and the migration of phagocytes such as polymorphonuclear leukocytes. The epithelium itself provides the first sensory line of defense and active sampling of commensals and pathogens. This occurs in specialized areas such as Peyer's patches in the ileum and solitary lymphoid nodules in the colon that are devoid of mucus on their surface. These lymphoid structures, which serve as inductive sites for the mucosal immune system, are overlaid by a follicle-associated epithelium that contains specialized translocating M cells. This system is likely to be involved in sustained lumenal sampling, to extend the host immune experience against any new incoming microorganism. Following their capture, commensal microorganisms never progress beyond mesenteric lymph nodes, whereas pathogens may take advantage of this route to invade the epithelium and possibly disseminate further into the organism, beyond the mesenteric lymph nodes. Microbes also can be sampled by alternative routes. Dendritic cells, for instance, can extend pseudopods between epithelial cells and sample bacteria in the lumen. Bacteria also can accidentally traverse the epithelial lining. These sampling conditions represent opportunities for the host to sense and recognize these microorganisms. It is generally believed that bacterial factors, also called pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs), are responsible for the molecular cross-talk established between the bacteria and the mucosal surface. PRRs are a combination of membrane-associated sensors, such as Toll-like receptors and cytosolic sensors such as nucleotide-binding oligomerization domain–like receptors. This, however, does not account for the peaceful state established with the commensals and the all-out war resulting from contact with bacterial pathogens, given that similar PAMPs generally are shared by both pathogenic and nonpathogenic microorganisms.

Commensals avoid inappropriate immune activation by engaging in cross-talk leading to tolerance taken in its general sense. Tolerogenic mechanisms encompass a complex array of characters involving the bacteria that lack virulence factors and whose certain PAMPs may be poorly agonistic for PRRs and the host. A certain degree of sequestration of PRRs leaves a low density of these sensors on the epithelial surface and a higher density in the crypts that are better defended against microbes.

Tolerance also involves an active process of regulation implicating ''tolerogenic'' dendritic cells and lymphocytes such as $CD25^{+}FoxP3^{+}$ Tregs, for which function largely may be induced and maintained by signals emitted by intestinal epithelial cells. Commensals largely are retained at a distance from the epithelial surface by the mucus layer, on the surface of which they form biofilm-like structures. They also lack attributes necessary to aggressively engage the epithelium, such as adhesins, invasins, and toxins. They still may be sensed by the epithelium, either through released PAMPs, (ie, released lipopolysaccharide sensed by Toll-like receptor-4, or muropeptide fragments sampled through dipeptide channels such as PepT1) or other components such as quorum-sensing molecules that can be sampled by epithelial cells through apical channels, including the organic cation transporter 2. Overgrowth of commensals, major imbalance between microbial species, and increased proximity to the epithelial surface may therefore be detected and the state of tolerance broken, leading to an inflammatory response.

Understanding the exact nature of this mechanism of balance is at the heart of the priorities in the field of innate immunity. Its rupture induces a switch from ''physiological'' to ''pathological'' inflammation that is characteristic of diseases such as IBD. Pathogens, conversely, are aggressive for the epithelial barrier. As shown in Figure 2, in comparison with commensals, they produce mucinases that hydrolyse mucus, thereby facilitating their access to the epithelial surface to which they can bind through adhesins, or they can penetrate through elaboration of invasins. Pathogens also secrete toxins, and gram-negative organisms possess type III and type IV secretion systems injecting effectors that allow further subversion of the epithelium. All together, the pathogenic phenotype is likely to directly trigger uncontrolled pathological inflammation by delivering PAMPs extremely close to, if not straight into, the epithelium and by possibly counteracting active tolerance mechanisms.

Recent evidence indicates, however, that bacterial pathogens often are able to modulate the intensity of the inflammatory response that they elicit. This is probably a matter of survival in a situation that would otherwise lead to their quick destruction, thereby leading to abortive infection.

Key References

Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med* 2006;203:2841–52.

Didierlaurent A, Sirard JC, Kraehenbuhl JP, Neutra MR. How the gut senses its content. *Cell Microbiol* 2002;4:61–72.

Elson CO, Cong Y, McCracken VJ, et al. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. *Immunol Rev* 2005;206:260–76.

Fujiya M, Musch MW, Nakagawa Y, et al. The *Bacillus subtilis* quorum-sensing molecule CSF contributes to intestinal homeostasis via OCTN2, a host cell membrane receptor. *Cell Host Microbe* 2007; 1:299–308.

Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004;4:478–85.

Sansonetti PJ, Di Santo JP. Debugging how bacteria manipulate the immune response. *Immunity* 2007;26:149–61.

Swidsinski A, Weber J, Loening-Baucke V, et al. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol* 2005;43:3380–9.

Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human–bacteroides thetaiotaomicron symbiosis. *Science* 2003;299:2074–6.

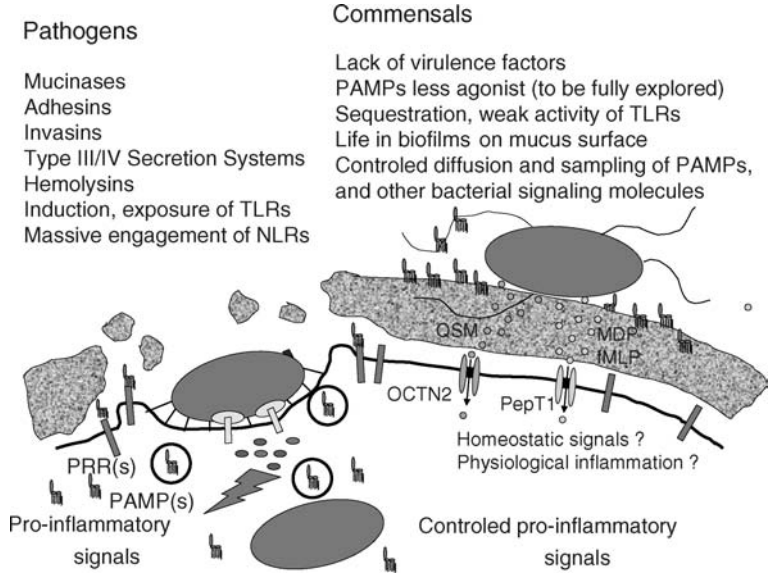

FIG. 2.

CELIAC DISEASE: SANDWICHED BETWEEN INNATE AND ADAPTIVE IMMUNE RESPONSES INDUCED BY GLUTEN

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The author reports no conflicts of interest.

Celiac disease (CD) is a disorder of the small intestine caused by intolerance to wheat gluten and related proteins in barley and rye. Common manifestations include chronic diarrhoea, abdominal pain, malnutrition, and failure to thrive. These symptoms result from an inflammatory immune response induced by gluten that leads to villous atrophy, hypertrophic crypts, and an intraepithelial lymphocyte infiltrate in the small intestine. The clinical picture normalises upon strict compliance to a gluten-free diet. CD can occur early in life, shortly after the introduction of gluten into the diet, but it also can develop much later in life. Also, disease severity varies significantly between patients, and only a minority present with the typical symptoms associated with CD. Although several screening studies have indicated that the prevalence of CD in the Western world is between 0.5% and 2%, only about 10% of patients are diagnosed.

There is a strong genetic predisposition to disease development: concordance in monozygotic twins is 80%, but it is only 10% in dizygotic twins. Moreover, the vast majority of patients with CD express histocompatibility leucocyte antigen (HLA)-DQ2, whereas HLA-DQ2–negative patients are usually HLA-DQ8 positive. Recently, the linkage between these HLA-class II molecules and gluten in CD development has become clear.

Gluten is a complex mixture of glutamine- and proline-rich proteins, a feature that is tightly linked to its disease-inducing capacity. Given its high proline content, gluten is highly resistant to enzymatic degradation in the intestine. Moreover, the high proline and glutamine content make it an ideal substrate for the enzyme tissue transglutaminase, which converts glutamine into the negatively charged glutamic acid. Given this introduction of negative charges, gluten peptides can bind with high affinity to HLA-DQ2 and HLA-DQ8 because these class II molecules have a preference for peptides with negative charges at anchor positions. It is now well established that gluten contains a large number of peptides that are substrates for tissue transglutaminase, bind to either HLA-DQ2 or -DQ8, and can trigger T cell responses (Fig. 3). Polyclonal T cell responses directed at multiple gluten peptides are therefore almost always observed in patients with CD.

In addition, gluten can active the innate immune system. Through an as yet unknown mechanism, gluten exposure leads to increased production of interleukin-15. This results in upregulation of the expression of NKG2D on intraepithelial lymphocytes and the induction of MICA on epithelial cells. Subsequently, the interaction between NKG2D and MICA leads to epithelial cell killing. Strikingly, the activation of the innate immune system is observed only in patients. In this respect, it is important to note that although the HLA-DQ locus is the major contributing genetic factor, CD inheritance does not follow a Mendelian segregation pattern. In all probability, multiple other genes, each with relatively weak effect, contribute to disease development. One of those gene products may mediate the activation of innate immunity in patients with CD.

In addition, an important role for environmental factors cannot be ignored because the concordance rate in monozygous twins is considerably less than 100%. The identification of these environmental factors and susceptibility genes will be required to fully understand disease aetiology, and this may provide diagnostic and prognostic markers. The recent identification of the interleukin-2/interleukin-21 locus as a susceptibility locus is a significant step forward in this respect.

The current treatment for CD consists of a lifelong gluten-free diet. Given the broad use of gluten in the food industry, this is not an easy task, and certainly poses some restrictions, especially social. Alternatives to a gluten-free diet are therefore being explored. These range from inhibitors of tissue transglutaminase and HLA-DQ-peptide binding to enzymatic degradation of gluten in the gastrointestinal tract.

Key References

Koning F. Celiac disease: caught between a rock and a hard place. *Gastroenterology* 2005;129:1294–301.

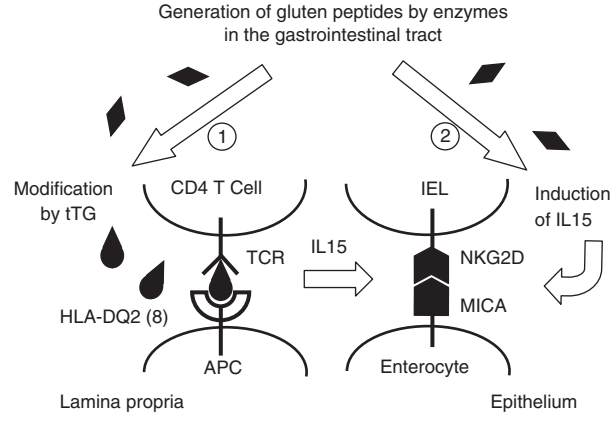

FIG. 3. Degradation of gluten generates fragments that can be modified by tissue transglutaminase (tTG). Subsequently, these fragments can bind to the disease-predisposing HLA-DQ2 or HLA-DQ8 molecules and trigger inflammatory T cell responses. Simultaneously, gluten can trigger interleukin (IL)-15 production, which leads to the upregulation of the NKG2D receptor on intraepithelial lymphocytes (IELs) and its ligand MICA on enterocytes, leading to enterocyte destruction. APC = antigen-presenting cell; TCR = T-cell receptor.

Sollid LM. Celiac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol* 2002;2:647–55.
Vader W, Stepniak D, Kooy Y, et al. The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T-cell responses. *Proc Natl Acad Sci USA* 2003;100:12390–5.

TWIN STUDIES IN IBD AND OTHER DISORDERS

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The author reports no conflicts of interest.

The 1988 Swedish study on the concordance rates among twins with IBD has been cited widely. The results of higher concordance rates among monozygotic twins compared with dizygotic twins, and the much higher concordance rate in Crohn disease (CD) compared with ulcerative colitis, formed our view of a stronger genetic component in CD than in ulcerative colitis. The pattern and magnitude of these results have since been replicated in other populations, as well as in a recent update of the Swedish twin registry. Moreover, they are in accordance with familial studies, which have been the backbone of different genetic studies.

The concordance rate of 50% among monozygotic twins with CD is high compared with other diseases, including different forms of cancer, in which a genetic component is established, such as multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis. Moreover, there also seems to be a remarkably high concordance rate among monozygotic twins with regards to phenotype at diagnosis and clinical course, indicating that genetics is also of importance in the disease course. However, a mutant allele of NOD2/CARD15 was as common in unaffected as in affected monozygotic twins with CD, a finding that implies other pathways underlying the high concordance in CD. Twin studies often are thought to be easy to interpret, but there are complications in study design that all too often are set aside. There are various problems in these studies, such as confounding by indication, lack of statistical power, and perhaps most important in CD, interactions with environmental factors such as smoking. Such an interaction also has been shown to be of importance in rheumatoid arthritis.

Key References

Halfvarson J, Bodin L, Tysk C, et al. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology* 2003;124:1767–73.
Jess T, Riis L, Jespersgaard C, et al. Disease concordance, zygosity, and NOD2/CARD15 status: follow-up of a population-based cohort of Danish twins with inflammatory bowel disease. *Am J Gastroenterol* 2005;100:2486–92.
Tysk C, Lindberg E, Järnerot G, Flodérus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988;29:990–6.

SESSION III

Impact of Microbial Colonization on Normal and Pathological Responses

Moderator: Lee Denson

EXISTING KNOWLEDGE OF THE HUMAN GUT MICROBIOTA

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The author reports no conflicts of interest.

The large intestine contains a diverse microbiota comprising several hundred different types of bacteria. Our understanding of its structure and function has changed dramatically over the last decade, due in large part to the introduction of molecular methods of microbial community analysis. Although the gut microbiota appears to be stable in the long term, and broadly similar in different people, significant differences can be seen in individual bacterial communities at the level of species and strains, whereas large variations in some populations can occur from day to day. Ecological factors that control the metabolic activities and composition of the microbiota are generally well understood. It is known, for example, that high species diversity is important in the maintenance of homeostasis and structural stability, and species loss as result of aging or antibiotic therapy impedes the ecosystem's ability to resist the ingress of pathogenic microorganisms. The normal gut microbiota has several important functions in host physiology and metabolism, and plays a key role in health and disease. Its physiological and biochemical activities are controlled by many factors, particularly diet and environment.

Although the pathogeneses of Crohn disease (CD) and ulcerative colitis (UC) still are not fully understood, it is well established that the microbiota plays a key role in the initiation and maintenance of the disease process. The microbiological composition of feces in patients with UC and CD has been studied for more than 50 years, and a number of reports have linked individual bacterial species to these diseases. However, there is no consistent evidence for the involvement of specific microbial agents, and evidence for a transmissible agent is weak. Changes certainly occur in bacterial population structure in the gut in IBD, but it is difficult to determine whether these manifestations are linked to disease etiology or are imposed by environmental transformations resulting from disease processes.

Increasingly, interest is focusing on the structure and function of mucosal communities in the gut and their role in IBD. Microbiological analysis of rectal tissue obtained from 9 patients with active UC and 10 controls yielded 72 different species of bacteria, with only 20 species being common to both subject groups. Putative pathogens belonging to the genus *Peptostreptococcus* were detected only in patients with UC, who also had more enterobacteria than the controls. However, detailed examinations of mucosal biofilms indicated that only differences in bifidobacterial populations—which are regarded as being protective organisms—were statistically significant, being 30-fold lower in UC samples.

A 4-week synbiotic feeding double-blind, randomized controlled trial using *Bifidobacterium longum* (probiotic component) and the prebiotic Synergy 1 subsequently was undertaken in patients with UC (n = 18) to determine whether bifidobacteria may be protective in this disease. Results from this pilot study showed that mucosal bifidobacteria increased 42-fold in patients given the synbiotic, compared with a 4.6-fold increase in the controls. This was associated with marked reductions in tumor necrosis factor-α and interleukin-1α in mucosal tissue, together with inducible human β-defensins in the synbiotic group. These antimicrobial peptides are secreted by inflamed gut epithelia, but not by infiltrating inflammatory cells in the mucosa, and were therefore useful markers of epithelial renewal, which was confirmed during histology. Sigmoidoscopy scores also improved in patients receiving the synbiotic, showing that this therapy resulted in amelioration of the full clinical appearance of chronic inflammation in these individuals.

Key References

Bartosch S, Fite A, Macfarlane GT, McMurdo MET. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients using real-time PCR and the effects of antibiotic treatment on the fecal microflora. *Appl Environ Microbiol* 2004;70:3575–81.

Furrie E, Macfarlane S, Kennedy A, et al. Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005;54:242–9.

Macfarlane S, Macfarlane GT. Bacterial diversity in the large intestine. *Adv Appl Microbiol* 2004;54:261–89.

INNATE IMMUNE RESPONSES TO COMMENSAL BACTERIA IN THE GUT EPITHELIUM

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The authors report no conflicts of interest.

The mammalian gut harbors a coevolved microbial consortium that makes critical contributions to host health. Despite the enormous number of bacteria in the luminal

compartment, microbial penetration of mucosal surfaces is relatively rare. We have shown that microbial colonization of germ-free mice triggers epithelial expression of RegIII-γ, a secreted C-type lectin. RegIII-γ binds intestinal bacteria but lacks the complement recruitment domains present in other microbe-binding mammalian C-type lectins. We have demonstrated that RegIII-γ and its human counterpart—hepatocarcinoma-intestine-pancreas/pancreatic-associated protein—are directly antimicrobial proteins that bind their bacterial targets via interactions with peptidoglycan carbohydrate. We propose that these proteins represent an evolutionarily primitive form of lectin-mediated innate immunity, and that they reveal intestinal strategies for maintaining symbiotic host–microbial relations.

Analysis of the host factors that regulate RegIII-γ expression has uncovered a mechanism of epithelial cell–intrinsic sensing of intestinal bacteria in vivo. We have shown that Paneth cells, specialized secretory epithelia of the small intestine, detect bacteria via cell-intrinsic MyD88-dependent Toll-like receptor signaling. Epithelial cell-intrinsic microbial sensing is triggered by bacterial penetration of the mucosal surface and activates a complex antimicrobial expression program. These results indicate that epithelial Toll-like receptors are key sensors of bacterial invasion of the intestinal mucosa, and reveal a direct dialog between the intestinal microbiota and the mammalian gut epithelium. Furthermore, our findings give new insight into how mammalian gut epithelia maintain homeostasis with resident intestinal bacteria.

CAPSULAR POLYSACCHARIDES OF SYMBIOTIC BACTERIA MODULATE IMMUNE RESPONSES DURING EXPERIMENTAL COLITIS

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The author reports no conflicts of interest.

The mammalian gastrointestinal tract harbors a complex ecosystem consisting of extraordinary numbers of resident bacteria in homeostasis with the host immune system. Here we demonstrate that during colonization of animals with the ubiquitous gut microorganism *Bacteroides fragilis* a bacterial polysaccharide (PSA) directs the cellular and physical maturation of the developing immune system. Comparison with germ-free animals reveals that the novel immunomodulatory activities of PSA during *B fragilis* colonization include correcting T cell deficiencies and T_H1/T_H2 imbalances, as well as directing lymphoid organogenesis. The mechanism of PSA activity requires antigen endocytosis by intestinal dendritic cells and presentation by major histocompatibility class II to signal $CD4^+$ T cell activation and cytokine production. Most significant, the benefit of this process for the host is demonstrated by PSA-conferred protection against experimental colitis induced by pathogenic bacteria.

The mammalian immune system has evolved an elaborate mechanism to delete or suppress inflammation against self-antigens, the details of which have been studied extensively for decades. The mechanism by which a host controls responses against encountered, nonpathogenic molecules (eg, commensal bacteria, food, inhaled antigens) is less well understood. For example, the gut microflora exposes every Toll-like receptor ligand to the mucosal epithelium without resulting in overt inflammation. In studies aimed at understanding this phenomenon, we used an animal model of colitis whereby inflammatory responses were directed against commensal bacteria, resulting in the onset of intestinal pathology and disease (wasting) in laboratory animals. Treatment with PSA completely protected animals from colitic disease, indicating that this molecule is involved in establishing immunological homeostasis of the gut. Pathological analysis demonstrates that PSA treatment completely ameliorates colonic inflammation and protects against wasting. Furthermore, colonization of laboratory animals with *B fragilis* also protects from the onset of colitis, whereas colonization with an isogenic derivative lacking only the production of PSA is unable to restore health. This research may ultimately lead to the development of therapeutic strategies to ameliorate human IBDs such as Crohn disease and ulcerative colitis.

In 1989 Strachan proposed the now famous ''hygiene hypothesis,'' whereby reduced exposure to infections in early childhood (owing to smaller families and improved living standards and personal hygiene) may result in an increased risk of developing allergic disease. This concept is illustrated by plotting the decrease or elimination of several infectious diseases and a coinciding increase in incidence of IBD, asthma, type 1 diabetes, and multiple sclerosis among western European countries. Furthermore, analysis of disease incidence among countries shows stark increases in multiple sclerosis and type 1 diabetes mellitus in those societies with improved medical care and hygiene. The control of infectious diseases—through vaccinations, sanitation, and antibacterial and antiviral therapies—represents one of the greatest accomplishments in medical science. However, most of these approaches do not discriminate between infectious and noninfectious microorganisms, and have undoubtedly led to changes in our association with the microbial world as a whole. The hygiene hypothesis does not address our most primary relationship with the multitudes of microbial species that we harbor as symbionts. Our studies recently have shown for the first time that symbiotic bacteria residing in the gastrointestinal

tract of mammals produce molecules that mediate the normal development of immune organs and healthy immune responses. Thus mammals, including humans, appear to have evolved an indispensable partnership with symbiotic bacteria that direct beneficial processes required for health.

Key References

Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347:911–20.

Strachan D. Hay fever, hygiene, and household size. *Br Med J* 1989;299:1259–60.

ROLE OF MICROBES IN THE ADAPTATION OF THE COLONIC EPITHELIAL PROGENITOR NICHE DURING INJURY

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The author reports no conflicts of interest.

The epithelial barrier is a single layer of nondividing, terminally differentiated cells that lines the inner surface of the cecum and colon. These cells undergo constant and rapid replacement during life. The source of this renewal is a proliferative population of colonic epithelial progenitors (ColEPs) located in the lower third of epithelial invaginations called crypts of Lieberkühn. ColEPs consist of tripotent stem cells that give rise to 2 sets of proliferative committed daughters; monopotent progenitors produce absorptive enterocytes, and bipotent progenitors give rise to secretory goblet and enteroendocrine cells. All 3 epithelial lineages continue their differentiation program as they migrate upward toward the luminal surface. Enterocytes are the predominant cell type that exits the crypt and forms the surface barrier epithelium. Most goblet cells remain in the crypt and their precise localization within this structure depends on their anatomic location along the length of the colon.

During homeostasis, Wnt and Notch signals are required to maintain ColEP proliferation while transforming growth factor-β/BMP act as a key negative regulator. Wnt, Notch, and Hedgehog signaling play a role in the modulating intestinal epithelial cell fate decisions. A key source for many of these signals is considered to be the ''stem cell niche.'' In the colon, the niche is composed of mesenchymal cellular networks of fibroblasts and blood vessels that envelop crypts and project toward the surface epithelium. Most colonic fibroblasts are myofibroblasts that express a subset of smooth muscle–specific proteins and form a prominent interlacing network of cells that forms a cuplike structure around each crypt. These cells may produce a variety of factors that support crypt proliferation and display an ability to migrate upward toward that luminal surface in concert with the overlying epithelial cells.

The epithelium that lines the inner surface of the intestine can be disrupted by a variety of environmental factors, ranging from pathogenic bacteria to drugs and irradiation. Injury may lead to circumscribed loss of the epithelium with formation of superficial erosions or more deeply penetrating ulcers. Repair of these lesions requires rapid recognition and response by the host because the intestinal lumen contains an abundant society of indigenous microbes, or microbiota, that can cause opportunistic infection if the epithelial barrier remains breached.

Injury requires the proper response from the ColEPs. Proliferation must be controlled to most effectively participate in wound repair, yet minimize damage to stem cell DNA. Recent studies suggest the niche components required for homeostasis are not the essential elements that control ColEP activity and proliferation during injury. The surprising feature of this system is that the microbiota itself participates in the decisions that are made regarding ColEP activity. Interestingly, it appears that members of the immune and mesenchymal systems act as cellular transceivers as they interpret signals from gut microbes and relay decisions to the adjacent ColEPs. These cells act as modifiers of the stem cell niche and modulate wound repair.

Key References

Brown SL, Riehl TE, Walker MR, et al. Myd88-dependent positioning of Ptgs2-expressing stromal cells maintains colonic epithelial proliferation during injury. *J Clin Invest* 2007;117:258–69.

Pull SL, Doherty JM, Mills JC, et al. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci USA* 2005;102:99–104.

Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, et al. Recognition of commensal microflora by TLRs is required for intestinal homeostasis. *Cell* 2004;118:229–41.

IMPACT OF GASTROINTESTINAL FLORA ON SYSTEMIC DISEASES

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Bengt Björkstén receives financial compensation as a consultant to BioGaia, a manufacturer of probiotic bacteria.

Microbes are essential for the development of normal immune regulation, and it has been suggested that a decrease in microbial stimulation associated with modern living could explain why allergic and autoimmune diseases are increasing. Recent epidemiological studies and experimental research suggest that the microbial

environment and exposure to microbial products in infancy modifies immune responses and enhances immune regulation and tolerance to ubiquitous antigens. The intestinal microbiota seems to play a particular role in this respect because it is the major external driving force in the maturation of the immune system after birth, and animal experiments have shown it to be a prerequisite for the development of normal oral tolerance.

The gastrointestinal tract of the newborn baby is sterile. Soon after birth, however, it is colonised by numerous types of microorganisms. Colonization is complete after approximately 1 week, but the numbers and species of bacteria fluctuate markedly during the first 3 to 6 months of life until the microbiota is established. Once established, there is a continuous interaction between the microbial flora and the host, making a dynamic ecosystem that is surprisingly stable under normal conditions. Environmental changes, such as a treatment period with antibiotics, only temporarily change the composition of the microbiota.

The gut microbiota is thus the quantitatively most important source of microbial stimulation and may provide a primary signal for driving the postnatal maturation of the immune system and the development of a balanced immunity. Thus, there is mounting evidence that commensal microbes acquired during the early postnatal period are required for the development of tolerance, not only to themselves but also to other antigens. For example, Th2-mediated immune responses are not susceptible to oral tolerance induction in germ-free mice. Furthermore, oral tolerance is induced only after the introduction of components of the normal microbiota to germ-free animals. It also is recognised that interaction with microbes, especially the normal microbial flora of the gastrointestinal tract, seems to be the principal environmental signal for postnatal maturation of T cell function in humans. Experimental and clinical studies also indicate that the quantity and function of T regulatory cells are affected by stimulation of gut microbes.

A number of diseases that seem to be related to a modern, affluent lifestyle may be associated with an altered gut microbiota via an effect on the developing immune system early in life. These ''immunologically mediated diseases of affluence'' include allergies, insulin-dependent diabetes mellitus and other autoimmune diseases, IBD, and—as suggested recently—some forms of obesity. Comparative studies performed in the 1990s in infants in Scandinavia and in formerly socialist eastern European countries with a lifestyle similar to that in Scandinavia some 30 to 40 years earlier revealed considerable differences in the composition of the gut microbiota. The composition in the latter countries was in many respects similar to what was reported in western Europe in the 1960s: in Scandinavia, there are now considerable differences as compared with the earlier studies. Since then, studies in several countries have revealed considerable differences in the composition of gut microbiota between healthy and allergic infants. Treatment studies with probiotic bacteria also have yielded encouraging results.

Much remains to be studied before the precise role of the gut microbiota in health and disease can be defined. It is reasonable, therefore, to devote more interest to our ''internal environment'' to obtain a better understanding of how the immune system is regulated and to search for environmental risk and protective factors related to immunologically mediated diseases of affluence. Such studies of the complex microbial ecology in the gut are likely to yield novel strategies for disease treatment and prevention in the future.

Key References

Guarner F, Bourdet-Sicard R, Brandtzaeg P, et al. Mechanisms of disease: the hygiene hypothesis revisited. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:275–84.

Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergic diseases. *J Allergy Clin Immunol* 2007;120:255–62.

Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027–31.

SESSION IV

Reciprocal Interactions Between Microbes and Immunity

Moderator: Edward Nieuwenhuis

THE INFLAMMASOME: A KEY PLAYER IN THE INFLAMMATION TRIGGERED IN RESPONSE TO BACTERIAL PATHOGENS

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The author reports no conflicts of interest.

Interleukin (IL)-1β and IL-18 are proinflammatory cytokines that are crucial for the immune responses to several pathogens, including intestinal pathogens such as *Salmonella typhimurium* and *Shigella flexneri*. Like most cytokines, IL-1β and IL-18 are regulated at the transcriptional level. However, these cytokines also require an additional processing step to be secreted. This maturation step is under the control of the cysteine protease caspase-1. Caspase-1 also can mediate cell death. Because of its dual role in triggering both the release of proinflammatory cytokines and cell death, caspase-1 is called an inflammatory caspase. Although the critical role of caspase-1 in the release of IL-1β and IL-18 in response to pathogens was widely known, how caspase-1 was activated remained unknown until the discovery of the inflammasome. The inflammasome is a multimolecular complex in which caspase-1 activation takes place after recognition of bacterial molecules and danger signals by nucleotide-binding oligomerization domain–like receptors (Fig. 4). We will present an overview of the inflammasome and its implication in several human inflammatory diseases. Next, we will address the role of the inflammasome in response to bacterial pathogens with a special emphasis on the response to the vacuolar pathogen *Salmonella typhimurium* and the cytosolic pathogens *Listeria monocytogenes* and *Francisella tularensis*. Finally, using recent data obtained in our laboratory, we will present the tight regulation of the inflammatory responses associated with the activation of the inflammasome.

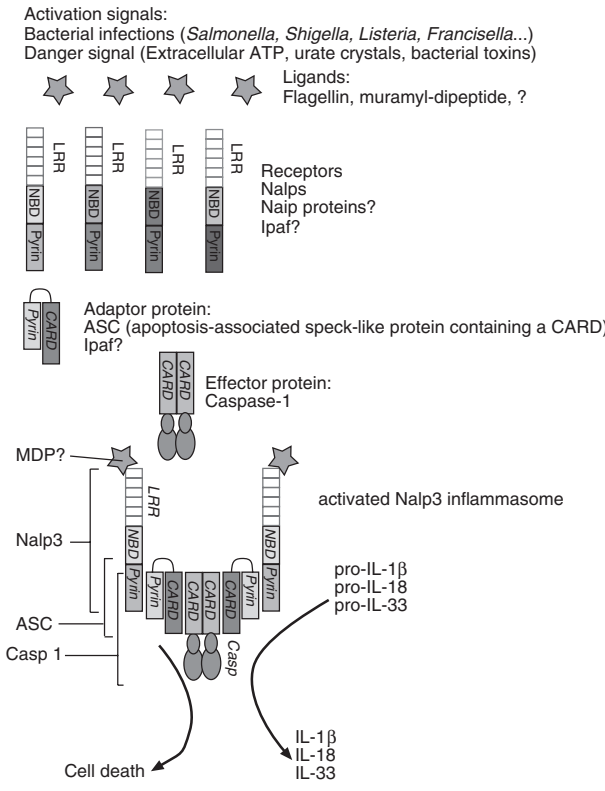

FIG. 4. The inflammasome is a multimolecular complex triggering caspase-1 activation. The inflammasome can be activated in response to a wide variety of bacterial infections and danger signals. Flagellin and muramyl dipeptide are ligands known to activate the inflammasome. The receptors of the inflammasome belong to a family of 14 proteins in humans called Nalp (NACHT-LRR– and Pyrin-containing proteins). Other nucleotide-binding oligomerization domain–like receptors such as proteins from the Naip family or Ipaf may be involved as sensors in the inflammasome. The adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) can bind both Nalp proteins and the inflammatory caspase and caspase-1, leading to the formation of an activated inflammasome, as exemplified with the Nalp3 inflammasome. Upon activation, caspase-1 maturation triggers the release of the proinflammatory cytokines interleukin (IL)-1α, IL-18, and IL-33, and in most cases cell death. Leucine-rich repeats (LRRs), nucleotide-binding domain (NBD), caspase recruitment domain (CARD), catalytic caspase domain (Casp), and Pyrin domain are shown.

Key References

Henry T, Brotcke A, Weiss DS, et al. Type I interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J Exp Med* 2007;204:987–94.

Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nature Rev Immunol* 2007;7:1–10.

Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. *Mol Cell* 2002;10:417–26.

PANETH CELLS, DEFENSINS, AND IBD

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Charles Bevins, together with other scientists at University of California, Davis, Dr. Margarete Fischer-Bosch Institute, and Ventria Biosciences, is a co-inventor of a patent pending on the use of defensins in IBD.

The intestine is a site of continuous and intimate contact with a complex, dense community of microorganisms, including hundreds of species of resident microflora and many transient microbes entering from food and waterborne sources. Many hypotheses on the pathogenesis of IBD hold that intestinal microbes contribute to disease progression in genetically susceptible individuals. What ordinarily is a balanced ecosystem among epithelial cells, immune cells, and resident flora is disrupted in IBD, resulting in chronic and relapsing inflammation of the intestinal mucosa. According to one general theory, an abnormal immune response, directed at an otherwise normal microflora, drives the chronic mucosal inflammation of IBD. A second school of thought proposes that an abnormality in the composition of the intestinal microbes, dysbiosis, drives the inflammation.

In the small intestine, Paneth cells (specialized secretory epithelial cells) produce high quantities of defensins and several other antibiotic peptides and proteins. Several functions have been ascribed to these Paneth cell effector molecules: shape composition of commensals, limit numbers of commensals, protect from pathogens, paracrine signaling, and stem cell protection. Data from murine models indicate that Paneth cell defensins play a pivotal role in defense from ingested pathogens in the intestinal lumen.

Recent studies in humans provide evidence that reduced Paneth cell defensin expression may be a key pathogenic factor in ileal Crohn disease (CD), a subgroup of IBD. Wehkamp et al analyzed ileal mucosal specimens using quantitative real-time polymerase chain reaction and found lower levels of Paneth cell α-defensin mRNA and protein in CD of the ileum, as compared with non-IBD controls. No significant decreases in 8 other Paneth cell products were seen, and the degree of inflammation could not explain the decrease in Paneth cell α-defensins. Interestingly, the specific decrease in α-defensins was more pronounced in the minority of patients that harbor a mutation in NOD2, the muramyl dipeptide recognition receptor. However, the underlying mechanism of α-defensin deficiency remains unclear. Wehkamp et al recently investigated the possible role of the Wnt signaling pathway, a key regulatory circuit for Paneth cell differentiation and α-defensin expression, in mediating the defensin deficit in the majority of patients who do not have mutations in NOD2. The data indicate the presence of reduced expression of the Wnt signaling transcription factor Tcf-4 in ileal CD, regardless of the degree of inflammation, but not in colonic CD or UC. Within specimens, the levels of Tcf-4 mRNA showed a high degree of correlation with both human defensin mRNAs (5 and 6). The question of why Paneth cell Tcf-4 expression is decreased is under investigation. Mechanistically, the link between reduced Paneth cell defensin expression and ileal CD pathogenesis may be a result of the weakened mucosal antimicrobial defense and/or alterations in the commensal microbiota.

Key References

Bevins C. Events at the host–microbial interface of the gastrointestinal tract V: Paneth cell α-defensins in intestinal host defense. *Am J Physiol* 2005;289:G173–6.

Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002; 347:417–29.

Porter EM, Bevins CL, Ghosh D, et al. The multifaceted Paneth cell. *Cell Mol Life Sci* 2002;59:156–70.

Salzman NH, Ghosh D, Huttner KM, et al. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. *Nature* 2003;422:522–6.

Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007;117:514–21.

Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal α-defensin expression. *Gut* 2004;53:1658–64.

Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell α-defensins in ileal Crohn's disease. *Proc Natl Acad Sci USA* 2005;102:18129–34.

Wehkamp J, Wang G, Kubler I, et al. The Paneth cell α-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007;179:3109–18.

Wilson CL, Ouellette AJ, Satchell DP, et al. Regulation of intestinal α-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999;286:113–7.

Kobayashi KS, Chamaillard M, Ogura Y, et al. NOD2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005;307:731–4.

EXPRESSION OF PHASE VARIABLE SURFACE MOLECULES OF *BACTEROIDES* SPECIES FROM HEALTHY AND CLINICAL STOOL

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The authors report no conflicts of interest.

The molecular mechanisms leading to the development of IBD are not fully understood, but the pathogenesis is known to involve host genetics, immune dysfunction, and intestinal microorganisms. Animal models have shown that the host's exaggerated immune response that leads to bowel inflammation is dependent upon the gut microbiota. Despite many important findings in recent years, it is not fully understood why the mucosal immune response in patients with IBD becomes overreactive to intestinal microbes.

The immunological aspects of IBD development have been studied extensively and continue to be an area of active research. The specific microbiological components that trigger IBD, however, have received less research attention. There is no microbial species or factor that has been implicated conclusively in IBD. Although

TABLE 1. *Surface molecules of Bacteroides fragilis that are phase variable dictated by DNA inversion of promoter regions*

Phase variable molecule/locus	Molecule	DNA invertase*	Relevant phenotype
PSA	Capsular polysaccharide	Mpi	Immunomodulatory
PSB	Capsular polysaccharide	Mpi	
PSC	Capsular polysaccharide	Mpi	
PSD	Capsular polysaccharide	Mpi	
PSE	Capsular polysaccharide	Mpi	
PSF	Capsular polysaccharide	Mpi	
PSG	Capsular polysaccharide	Mpi	
PSH	Capsular polysaccharide	Mpi	
MCR1	Unknown	Mpi	
MCR2	Unknown	Mpi	
MCR3	Unknown	Mpi	
MCR4	Unknown	Mpi	
EPS	Extracellular polysaccharide	Tsr19	Large capsule
AapA-AapH	Surface lipoproteins	AapI (Tsr15)	Adherence, biofilm
BF2866-BF2876	Surface lipoproteins	Tsr25	
BF4085-BF4087	Surface lipoproteins	Tsr26	

* Site-specific recombinase that mediates inversion of the promoter of the respective regions.

there may not be 1 specific microbial factor that triggers IBD, bacterial surface molecules likely are important in this process. The importance of bacterial surface molecules in mediating crucial interactions with the host is well established for pathogenic bacteria. In contrast, little is known about how the surface molecules of mutualistic organisms contribute to their interaction with the host in commensal, symbiotic, or potentially pathogenic relations. Commensal intestinal bacteria are believed to associate with the surface mucus rather than directly binding to enterocytes, as occurs with many enteric pathogens. This passive mucosal association of commensal organisms would not trigger the aggressive immune response mediated by intestinal pathogens.

Of the hundreds of bacterial species that inhabit the gut, *Bacteroides* species are among the most abundant and are important human symbionts of this ecosystem. *Bacteroides* species colonize the infant as early as 1 week after birth and are consistently present in the population by 1 year of age. *Bacteroides* spp. are unusual among commensal organisms in that they are able to rapidly change their surfaces. In *B fragilis*, the most studied of these organisms, these changes include the expression of many classes of surface proteins, adherence factors, and multiple capsular and extracellular polysaccharides (Table 1). The variable expression of these surface molecules leads to dramatic differences in antigenicity, immunogenicity, and phenotypic effects in the intestine. One phase variable molecule of *B fragilis* that has been studied leads to massive bacterial aggregation, surface attachment, biofilm formation, and adherence to intestinal enterocytes. Another phase variable molecule is involved in immune regulation in the mammalian intestine. Alteration in the expression of these physiologically relevant surface molecules could lead to the expression of pathogenlike phenotypes and immune dysregulation in a genetically susceptible host. Comparison of the expression potential of these phase variable molecules from IBD stool versus control stool may help to identify potential bacterial surface molecules of indigenous intestinal organisms that are associated with disease.

Key References

Krinos CM, Coyne MJ, Weinacht KG, et al. Extensive surface diversity of a commensal microorganism by multiple DNA inversions. *Nature* 2001;414:555–8.

Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122:107–18.

Palmer C, Bik EM, Digiulio BD, et al. Development of the human infant intestinal microbiota. *PLoS Biol* 2007;5:e177.

Weinacht KG, Roche H, Krinos CM, et al. Tyrosine site-specific recombinases mediate DNA inversions affecting the expression of outer surface proteins of *Bacteroides fragilis*. *Mol Microbiol* 2004;53:1319–30.

SESSION V

The Immune Response in Early-onset Inflammation

Moderator: Salvatore Cucchiara

ROLE OF NEUTROPHILS ON GENETIC DISORDERS OF PHAGOCYTE FUNCTION LEADING TO IBD

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The author reports no conflicts of interest.

Neutrophil responses are initiated as circulating neutrophils flowing through the postcapillary venules detect low levels of chemokines and other chemotaxis substances released from the site of infection. These soluble effectors of inflammation trigger subtle changes in the array and activity of surface molecules on both endothelial cells and neutrophils. Once the neutrophils are in the tissue, microbicidal activity takes place, which is dependent on granule fusion to the phagosome and generation of reactive oxygen products. These events jointly serve to enhance breakdown or clearance of pathogens from the site of infection accompanied by inactivation of chemotactic factors, which serves to terminate the process of neutrophil influx leading to attenuation of the inflammatory process. Neutrophils then undergo apoptosis, preventing injury to host tissue by containing activated microbicidal proteases within senescent neutrophils.

Neutropenic enterocolitis is an acute syndrome characterized by cecal and ascending colon inflammation that may progress to necrosis and perforation. It most often is associated with leukemia, but also been has described in 3 rare genetic disorders of neutropenia. These disorders include severe congenital neutropenia, glycogenosis type 1B, and cyclic neutropenia. Cyclic neutropenia is a rare hematological disorder characterized by recurrent fevers, mouth ulcers, and infections attributed to regularly recurring severe neutropenia. Characteristically, the mouth ulcers are deep and painful, and often last $\geq$1 week. Colonic ulcers may occur during the neutropenic period, similar to the patient's mouth ulcers, and often result in bacteremias due to clostridial species and gram-negative organisms. The neutropenic disorders have a number of features in common with that seen in chronic granulomatous disease (CGD). These include fine granular brown-pigmented macrophages, a paucity of neutrophils, and clusters of epithelioid histiocytes.

CGD results from defects in the superoxide-generating phagocyte oxidase causing recurrent infections with catalase-positive organisms, which leads to excessive inflammation and granuloma formation. Mutations in $gp91^{phox}$ are responsible for X-linked CGD comprising approximately two thirds of CGD cases. Inflammatory gastrointestinal involvement in CGD has been recorded in 17% to 33% of patients, the majority of whom have the X-linked inheritance. It is often a chronic relapsing and remitting condition with 2 overlapping phenotypes, ulcerative colitis and Crohn disease. In general, the gastrointestinal pathology is more similar to Crohn disease than ulcerative colitis, with patchy distribution and well-formed granuloma. In a large study from the National Institutes of Health, it was reported that the median age of initial gastrointestinal manifestation was 5 years. Abdominal pain was the most frequent symptom, and hypoalbuminemia was the most frequent laboratory finding. Prednisone controlled symptoms and signs in the majority of patients, but relapse of symptoms occurred in 71%.

The pathophysiology of CGD may include aberrant functioning T lymphocytes and neutrophils. The phagocyte oxidase is present at low levels in T lymphocytes and its absence causes a switch of T lymphocytes to a Th1 cytokine pattern upon activation; this may explain why patients with CGD appear to be at increased risk for a variety of Th1-type–specific immune diseases, such as Crohn disease and juvenile rheumatoid arthritis. Additionally, defective antibody-dependent endocytosis can be associated with CGD. The inability to ingest immune complexes by CGD neutrophils may contribute to the development of the immune complex disease leading to heightened states of inflammation and the failure to undergo apoptosis.

Key References

Malech HL, Hickstein DD. Genetics, biology, and clinical management of myeloid call primary immune deficiencies: chronic granulomatous disease and leukocyte adhesion deficiency. *Curr Opin Hematol* 2007; 14:29–36.

Marciano BE, Rosenzweig SD, Kleiner DE, et al. Gastrointestinal involvement in chronic granulomatous disease. *Pediatrics* 2004; 114:462–8.

Petty HR, Francis JW, Boxer LA. Deficiency in immune complex uptake by chronic granulomatous disease neutrophils. *J Cell Sci* 1988;90:425–32.

INTESTINAL EPITHELIAL CELLS CONTROL DENDRITIC CELL FUNCTION

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The author reports no conflicts of interest.

The gut immune system is exposed continuously to intestinal microflora, but also to pathogens accidentally

ingested with food. The role of the intestinal microflora in the development and correct functionality of the immune system is becoming increasingly evident. A perturbation of the gastrointestinal microflora or unwanted immune responses to this flora have been demonstrated to play a critical role in the pathogenesis of IBD in experimental animal models. Thus, the gut immune system has the difficult task of discerning between harmless microorganisms that are required for the correct functionality of the gut and potentially harmful bacteria.

A first line of defense toward putative "dangerous" microorganisms is provided by intestinal epithelial cells (IECs), which present a physical, chemical, and electric barrier to microbial entrance. Bacteria can gain access across mucosal surfaces either through M cells or through a recently described mechanism that is mediated by dendritic cells (DCs). Both portal entries are not necessarily restricted to invasive/pathogenic bacteria. Even though mucosal DCs are exposed to activating Toll-like receptor ligands, the inflammatory response is kept at bay.

How the mucosal immune system can limit the initiation of inflammatory reactions is not known. We show that at steady state, IECs condition anti-inflammatory DCs through the constitutive release of thymic stromal lymphopoietin (Fig. 5). IEC-conditioned DCs, although phenotypically activated by bacteria, lose the ability to produce interleukin-12. IEC-conditioned DCs release interleukin-6 and interleukin-10, but not IL-12, and polarize T cells toward a mucosal noninflammatory T helper-2 phenotype or T regulatory cells, even after a strong Th1 inducer such as *Salmonella typhimurium*. Only DCs not undergoing IEC conditioning and able to directly contact bacteria can initiate protective Th1 T cell responses. This control is lost in patients with Crohn disease. Indeed, IECs isolated from patients with Crohn disease are unable to control DC function and DCs become highly immunostimulatory. Furthermore, in 75% of the patients IECs do not express thymic stromal lymphopoietin constitutively. We also have analyzed the ability of DCs derived from peripheral blood monocytes of patients with Crohn disease to respond to gram-positive and gram-negative bacteria and found that they had altered bacterial handling. This correlated with the recently described disease-associated NOD2 mutations. In particular, we observed that 1 of 3 more common variants of the NOD2 gene (L1007fs) mutation resulted in a loss of function response to gram-positive and gram-negative bacteria, whereas the other 2 mutations conferred a gain of function also at baseline.

We also show that DCs play a major role in driving the development of regulatory T cells, as well as in facilitating the generation of systemic immunoglobulin G responses. Bacteria entering the gut via the DC-mediated mechanism can gain access to the mesenteric lymph nodes and the spleen to initiate a systemic immunoglobulin G response. On the contrary, bacteria entering via the Peyer patches can induce the development of mucosal immunoglobulin A responses that are protective against subsequent challenges via the oral route of administration.

These data suggest that the intestinal immune homeostasis is a highly regulated process that is initiated at the level of epithelial cells and is maintained at the level of DCs.

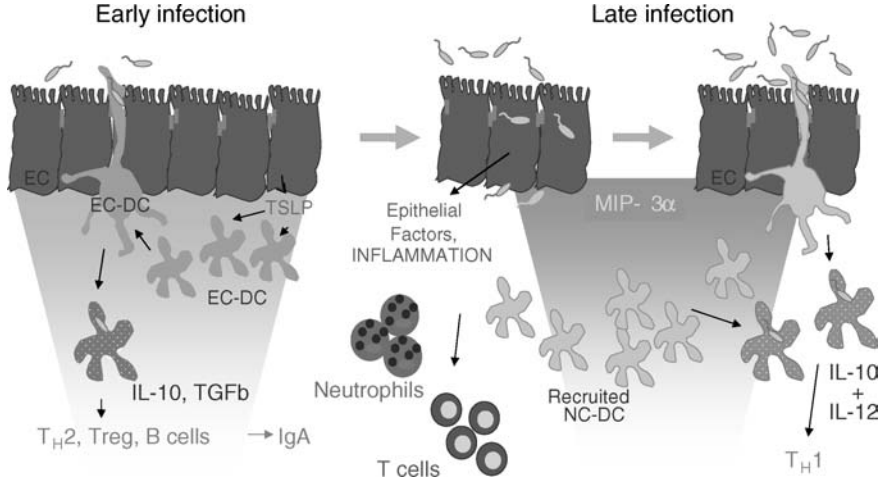

FIG. 5. Intestinal epithelial cells control dendritic cell function. Early infection: Resident dendritic cells (EC-DC) are "conditioned" by intestinal epithelial cell (EC)-derived factors, including thymic stromal lymphopoietin (TSLP) and drive the development of Th2 and Treg cells even in response to Th1-inducing bacteria. Conditioned DCs also can favor the development of immunoglobulin A–producing B cells. Late infection: At later time points, invasive bacteria such as *Salmonella* can drive the release of inflammatory mediators by ECs and of chemokines able to attract immature DCs. These recruited DCs are not "conditioned" and can initiate inflammatory responses to the encountered bacteria.

Key References

Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004;4:478–85.

Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;2:361–7.

Rimoldi M, Chieppa M, Salucci V, et al. Intestinal immune homeostasis is regulated by the crosstalk between epithelial cells and dendritic cells. *Nat Immunol* 2005;6:507–14.

Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. *Ann Rev Immunol* 2002;20:495–549.

SPECIFYING HELPER T CELL FATES DURING IMMUNITY

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The authors report no conflicts of interest.

Heterogeneity is a hallmark of antigen-specific T cells. $CD4^+$ T cells make effector choices to become T helper (Th1), Th2, or Th17 cells, each of which serves a distinct function in host defense (Fig. 6). Th1 cells facilitate the eradication of intracellular pathogens, Th2 cells aid in the elimination of parasitic worms, and the recently discovered Th17 subset appears to provide immunity against extracellular bacteria and fungi. Rather than becoming effector subsets, $CD4^+$ T cells also can become antigen-specific regulatory (adaptive Treg) cells, which may serve to dampen the extent of inflammation caused by their effector siblings. Memory T cells are also heterogeneous, with effector memory cells that provide immediate protection upon re-encountering pathogens and central memory cells that are capable of efficient homeostatic renewal.

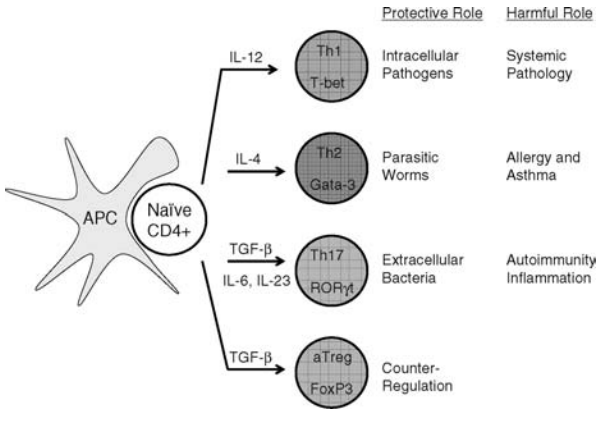

FIG. 6. Heterogeneity in the $CD4^+$ T cell response. Inductive cytokines, key transcription factors, and major functions of Th1, Th2, Th17, and adaptive regulatory (aTreg) cells.

Although T cell diversity is a central feature of immune responses, it remains unknown how this heterogeneity is achieved. One model to explain cell fate heterogeneity in the immune response is that several naïve T cells undergo distinct signaling events, with each cell giving rise to homogeneous progeny. In such a model, for instance, the first naïve T cell to encounter an antigen-presenting cell (APC) in the lymph node may give rise to effector progeny and undergo more massive expansion. Then, when a later-arriving naïve T cell reaches the draining lymph node, it may encounter a more exhausted APC, which now instructs it to be the precursor of memory cells. In a ''1 cell, 1 fate'' model, heterogeneity in cell fate is generated by the recruitment of several naïve T cells, leading to diversity at the population level.

Alternatively, a single naïve T cell may divide and give rise to progeny with different fates. In a ''1 cell, multiple fates'' model, heterogeneity could be generated during the immune response by the recruitment of a single cell. Such a model, however, would necessitate a mechanism for diversification. In recent years, it has become increasingly appreciated that deterministic mechanisms exist throughout metazoan development to confer disparate fates among daughter cells, using a strategy called asymmetric cell division. During asymmetric cell division, fate determinants are segregated to 1 side of the plane of division; the unequal inheritance of critical proteins results in different fates for the daughters.

Recent work in our laboratory suggests that asymmetric cell division may represent a mechanism to ensure that appropriate diversity of cell fate arises from the descendants of a single lymphocyte during an immune response. Dividing $CD8^+$ T cells, responding to a microbial pathogen, asymmetrically segregate proteins with known roles in signaling and cell fate specification. This asymmetry appears to be coordinated by the prolonged interaction between the T cell and its APC before division. Furthermore, phenotypic and functional analyses suggest that the resulting daughters are differentially fated into the effector and memory lineages.

Like $CD8^+$ T cells, naïve $CD4^+$ T cells also appear to undergo asymmetric division during an immune response. The fact that $CD4^+$ T cells are confronted by multiple fate choices beyond effector versus memory lineage alternatives, however, raises the question of what cell fates may be specified by asymmetric division. The finding that $CD4^+$ T cells may re-engage APCs after they have divided suggests the possibility that several rounds of asymmetric division may occur, resulting in an array of lineage-committed progenitors. For example, the receptor for interferon-γ appears to be unequally partitioned in naïve $CD4^+$ T cells preparing for their first division in vivo. Division could give rise to 1 daughter committed to the Th1 lineage, with the other daughter retaining

plasticity. With subsequent divisions, daughters could further diversify as Th2-, Th17-, or adaptive regulatory T cell–committed progenitors, as well as precursors of the memory lineage. Pathogen-related signals, such as the presence or absence of polarizing cytokines, may subsequently determine the final outcome.

Key References

Celli S, Garcia Z, Bousso P. CD4 T cells integrate signals delivered during successive DC encounters in vivo. *J Exp Med* 2005;202: 1271–8.

Chang JT, Palanivel VR, Kinjyo I, et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* 2007;315:1687–91.

Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002;2:933–44.

EARLY AND LATE GUT IMMUNE RESPONSES IN IBD

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IBD includes Crohn disease and ulcerative colitis. Both conditions are characterized by periods of remission that are interrupted by acute flares. There is increasing evidence derived from studies in humans and animals suggesting that induction of active disease and perpetuation of chronic intestinal inflammation are immunologically distinct phenomena. Studies in humans, for example, have shown that various cytokines predominate in early, as opposed to late, Crohn disease. Similarly, immunomodulation of early IBD in pediatric patients results in longer periods of remission than those reported for adults. These differences may be explained by the fact that the dysregulated immune response occurs at an earlier stage in children, rendering it more susceptible to definitive reversion by aggressive disease-modifying therapies.

This concept has been further investigated in studies using animal models of IBD, including the interleukin-10–deficient mouse model of colitis and the senescence-accelerated mouse P1/YitFc mouse, a model of chronic ileitis that has many similarities to Crohn disease. For example, spontaneous ileitis in senescence-accelerated mouse P1/YitFc mice commences with an induction phase that precedes the development of histological disease. At this stage, disease is strictly Th1-polarized, with early increases in mucosal expression of interferon-γ and tumor necrosis factor. However, after ileitis enters the chronic phase, the mucosal phenotype shifts toward a mixed Th1/Th2 pattern. In addition to interferon-γ and tumor necrosis factor, elevation of mucosal interleukin-13 and interleukin-5 is also observed.

Similar phase-specific changes occur in the expression of adhesion molecules and their ligands. During the induction phase, lymphocytes that are similar to those in the healthy mucosa are recruited through homeostatic mechanisms. On commencement of the chronic phase of disease, these homeostatic pathways are substantially upregulated. In addition, alternative pathways that involve proinflammatory chemokines, adhesion molecules, and integrins are triggered at this time. This results in heavy infiltration of the lamina propria with inflammatory cells. This presentation will focus on the important implications for therapy related to immunological modulation of early versus late gut inflammatory responses.

Key References

Bamias G, Martin C, Mishina M, et al. Proinflammatory effects of TH2 cytokines in a murine model of chronic small intestinal inflammation. *Gastroenterology* 2005; 128:654–66.

Desreumaux P, Brandt E, Gambiez L, et al. Distinct cytokine patterns in early and chronic ileal lesions of Crohn's disease. *Gastroenterology* 1997;113:118–26.

Markowitz J, Grancher K, Kohn N, et al. A multicenter trial of 6-mercaptopurine and prednisone in children with newly diagnosed Crohn's disease. *Gastroenterology* 2000;119:895–902.